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Progesterone increases susceptibility of gilts to uterine infections after intrauterine inoculation with infectious bacteria[1]

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ABSTRACT: In cattle and sheep, a progestogenated uterus is susceptible to infections, but this is not well documented for pigs. Therefore, the effects of day of the estrous cycle and progesterone on the susceptibility to uterine infections were evaluated. Gilts (n = 5 per group) were assigned to treatments in 2 × 2 factorial arrays. In Exp. 1, day of cycle and bacterial challenge were main effects. On d 0 or 8, uteri were inoculated with either 70×10^7 cfu of *Escherichia coli* and 150×10^7 cfu of *Arcanobacterium pyogenes* in PBS or with PBS. In Exp. 2, ovariectomy (OVEX) and progesterone treatment were main effects. On d 0, gilts were ovariectomized or a sham procedure was performed. After surgery, gilts received i.m. injections of progesterone (10 mg/5 mL) or 5 mL of safflower oil diluent twice daily. On d 8, gilts were inoculated with the same doses of bacteria as in Exp. 1. In Exp. 1 and 2, vena caval blood was collected for 4 d, after which uteri were collected. Sediment and ability to culture *E. coli* and *A. pyogenes* from uterine flushings were used to diagnose infections. Differential white blood cell counts and lymphocyte response to concanavalin A (Con A) and lipopolysaccharides (LPS) were used to measure lymphocyte proliferation. Progesterone, estradiol-17β, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and prostaglandin E_2 (PGE_2) were measured in vena caval blood. In Exp. 1, d-8 gilts receiving bacteria developed infections, but d-0 gilts receiving bacteria did not. Daily percentages of neutrophils and lymphocytes changed ($P < 0.05$) with cycle day and bacterial challenge. Basal- and Con A-stimulated lymphocyte proliferation were greater ($P < 0.05$) for d-0 than for d-8 gilts. Concentrations of $PGF_{2\alpha}$ ($P < 0.01$) and PGE_2 ($P < 0.05$) increased after bacterial challenge, regardless of stage of the estrous cycle at the time of inoculation. In Exp. 2, OVEX decreased ($P < 0.001$) and progesterone treatment increased ($P < 0.001$) progesterone concentrations, and OVEX decreased ($P < 0.01$) estradiol-17β. Gilts with ovarian and/or exogenous progesterone developed infections. Daily percentages of neutrophils and lymphocytes changed in response to OVEX, and neutrophils changed ($P < 0.05$) in response to endogenous and exogenous progesterone. Lymphocyte proliferation in response to Con A and LPS increased ($P < 0.05$) with OVEX and decreased ($P < 0.05$) with progesterone treatment. We conclude that endogenous and exogenous progesterone reduce the ability of the uterus in gilts to resist infections.

Key Words: Gilts, Infections, Progesterone, Uterus

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Introduction

Nonspecific uterine infections reduce reproductive efficiency of ruminants (for reviews, see Arthur et al., 1989; Lewis, 1997), but the incidence and effects of uterine infections in pigs are unreported. In cows and sheep, impaired or down-regulated neutrophil and lymphocyte functions seem to increase susceptibility to uterine infections (Lewis, 1997). This has not been evaluated for pigs. In ruminants and rabbits, resistance to uterine infections is greatest at estrus and least during the luteal phase (Black et al., 1953a,b; Rowson et al., 1953; Ramadan et al., 1997). Patterns of resistance and susceptibility to infections are closely linked to changes in progesterone and estrogen concentrations during the estrous cycle (Ramadan et al., 1997). Typically, increased concentrations of progesterone down-regulate immune cell functions, whereas reduced progesterone and increased estrogen concentrations up-regulate them. Unlike in ruminants, bacteria in pigs may be able to survive the follicular phase and proliferate, leading to infections during the luteal phase that are associated with increased embryonal loss (Scofield et al., 1974) and which indicate that mechanisms controlling uterine immune cell activity may be different in pigs and ruminants.

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Thus, this study was conducted with gilts to evaluate 1) immune cell responses to bacterial challenge during the follicular and luteal phases of the estrous cycle, 2) the ability of progesterone to modulate the uterine response to bacterial challenge, and 3) the value of pigs for detailed studies of uterine infections.

Materials and Methods

General

Gilts that were 9 to 12 mo of age, produced at the Virginia Polytechnic Institute and State University (Virginia Tech) Swine Center, and had had at least two consecutive estrous cycles of 19 to 22 d in duration were used for this study. All of the gilts were healthy and had no history of uterine infections.

Estrus Synchronization

Beginning 14 d after detection of estrus, 15 mg of altrenogest, which was mixed with the feed, was administered once daily for 10 d. Gilts were housed in stalls and fed individually. After removal of altrenogest from diets, boars were used twice daily to check for signs of estrus. Lordosis was considered the definitive sign of estrus. Gilts that were not detected in estrus within 6 d after altrenogest removal were not assigned to the experiment.

Vena Caval Catheterization and Blood Collection

Approximately 12 h before intrauterine inoculations in each gilt, a polyvinyl catheter was positioned in the vena cava via a saphenous vein at a point cranial to the site of entry of uteroovarian blood (Benoit and Dailey, 1991). To determine catheter position, blood from four regions of the vena cava (i.e., 50, 55, 60, and 65 cm from the point of entry into the saphenous vein) was assayed for progesterone or estradiol-17β, depending on day of the estrous cycle. The position with the greatest concentration of the assayed steroid hormone was considered to be the point cranial to the site of entry of uteroovarian blood. Blood collected from that region of the vena cava was presumed to either contain lymphocytes that had filtered through the uterus and/or be affected by the uterine environment.

Beginning 12 h after catheterization, vena caval blood was collected at 12-h intervals (a 12-mL sample at each time) for 4 d and transferred into heparinized Vacutainer tubes (Becton Dickinson, Rutherford, NJ). The heparinized samples were used to prepare blood for differential white blood cell counts (**WBC**) and to separate lymphocytes for lymphocyte proliferation assays. Differential WBC counts and lymphocyte proliferation assays were used to provide estimates of different aspects of WBC activity. Plasma was separated from samples and stored at –20°C until progesterone, estradiol-17β, $PGF_{2\alpha}$, and PGE_2 were quantified. Equal volumes of plasma from the two samples taken 12 h apart each day were pooled to provide average daily concentrations (Fortín et al., 1994). Wright stain and a light microscope with an oil-immersion objective were used for differential WBC counts (lymphocytes, neutrophils, monocytes, eosinophils, and basophils per 100 WBC), which were based on morphological characteristics of the cells.

Intrauterine Bacterial Inoculations

The strains of *Arcanobacterium pyogenes* and *Escherichia coli* used for this study were isolated from a cow with a uterine infection at the Virginia Tech Dairy Center. These bacteria have been used to produce endometritis in cows, sheep, and pigs (Del Vecchio et al., 1992; Ramadan et al., 1997; Wulster-Radcliffe, 2000). Both strains of bacteria were purified and stored in a skim milk broth medium at –20°C until they were used to prepare intrauterine inoculations (Ramadan et al., 1997). On the basis of a dose response curve (unpublished results), each inoculation contained 70×10^7 cfu of *E. coli* and 150×10^7 cfu of *A. pyogenes*. The suspension of bacteria was injected into the uterine horn more easily visualized with a laparoscope (Ramadan et al., 1997).

Lymphocyte Separation and Lymphocyte Proliferation Assay

Heparinized blood (12 mL) was centrifuged at 2,800 $\times g$ for 20 min at 4°C. The buffy coat was mixed with 4 mL of Hank's Balanced Salt Solution (**HBSS**) at pH 7.4 (Gibco BRL, Grand Island, NY). The mixture was layered atop 4 mL of Ficoll-Paque (Sigma Chemical, St. Louis, MO) and centrifuged at 400 $\times g$ for 30 min at room temperature. The lymphocyte-containing portion was transferred to sterile culture tubes. The suspension was centrifuged at 1,000 $\times g$ for 10 min at room temperature. The supernatant was removed, and the lymphocytes were washed twice with HBSS (5 mL/wash). The cells were then suspended in complete RPMI-1640 medium (Gibco) that contained fetal bovine serum (0.1 mL/mL; Gibco), penicillin (100 IU/mL; Sigma), and streptomycin (100 mg/mL; Sigma). The number of live lymphocytes was determined using a hemocytometer and a trypan blue dye-exclusion procedure. The final concentration of live cells in RPMI-1640 was adjusted to 1×10^6/mL of RPMI-1640.

The lymphocyte proliferation assay was similar to one described in Burrells and Wells (1977). Lymphocytes (1×10^5 live cells/100 μL of RPMI-1640) from each vena caval sample were cultured in 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ). Mitogenesis was stimulated with concanavalin A (**Con A**; stimulates T lymphocytes; 1 μg/100 μL of RPMI-1640; Sigma) or lipopolysaccharides (**LPS**; stimulates B lymphocytes; 0.5 μg/100 μL of RPMI-1640; Sigma), or cells were left unstimulated (100 μL of RPMI-1640) so that

unstimulated mitogenesis could be estimated. Incubation treatments were in triplicate, with a total of 200 μL of medium in each microtiter well.

Microtiter plates were held at 37° for 48 h in a humidified chamber with an atmosphere of 5% CO_2 in air. [^{3}H]Thymidine (1 μCi; specific radioactivity 4 Ci/mmol; ICN Radiochemicals, Irvine, CA) in 10 μL of RPMI-1640 was then added to each well, and the plates were maintained under the same conditions for another 16 h. At the end of culture, lymphocyte viability was determined with a trypan blue dye-exclusion procedure, and cells were transferred to fiberglass filters (Whatman, Maidstone, England). Filter disks corresponding to each well were transferred to separate scintillation vials, and lymphocytes were solubilized to release [^{3}H]thymidine. Disintegrations per minute were determined with a liquid scintillation counter and used to calculate the picomoles of [^{3}H]thymidine incorporated into newly synthesized DNA.

Immunoassays

Radioimmunoassays were used to quantify progesterone, estradiol-17β, and PGE_2. For catheter positioning and assay of samples, kits with [^{125}I]progesterone and [^{125}I]estradiol-17β ligands (Diagnostic Products, Los Angeles, CA) were used. All samples for progesterone and estradiol-17β after final catheter positioning were evaluated for both experiments in a single progesterone (CV = 8.6%) and a single estradiol-17β (CV = 9.7%) assay. Concentrations of PGE_2 were determined with a RIA that includes tritiated ligand (Lewis et al., 1978). The PGE_2 intra- and interassay CV for Exp. 1 were 11.2 and 23.1%, respectively, and for Exp. 2, they were 9.7 and 21.4%, respectively.

Concentrations of $PGF_{2\alpha}$ were determined with an EIA (Del Vecchio et al., 1992; Fortín et al., 1994). The intra- and interassay CV for $PGF_{2\alpha}$ for Exp. 1 were 7.8 and 24.2%, respectively, and for Exp. 2, they were 5.9 and 26.2%, respectively.

To avoid confounding the concentrations of a compound for a gilt with the interassay variation, a randomized block procedure (Cochran and Cox, 1957) was used to determine the assignment of samples to individual PGE_2 and $PGF_{2\alpha}$ assays. For each prostaglandin measured, all of the samples from a gilt were randomized and evaluated in the same assay.

Diagnosis of Uterine Infections

All gilts were slaughtered at the end of the sampling period. To determine whether a gilt had a uterine infection, the uterus was collected, contents were flushed out with 0.9% NaCl solution (wt/vol), samples were cultured for bacteria, and flushings were centrifuged. Clear uterine flushings, small amounts of sediment (<5% by volume in the flushings), and the inability to culture *A. pyogenes* and *E. coli* from the flushings were signs that the uterus was not infected. Cloudy uterine flushings, large amounts of sediment (>5%, but usually >40%, by volume in the flushings), and the ability to culture *A. pyogenes* and *E. coli* from the flushings were signs of infection.

Experimental Protocols

Experiment 1. The major objectives for Exp. 1 were to determine 1) the effects of stage of the estrous cycle on the response of the uterus to intrauterine inoculation with a combination of *A. pyogenes* and *E. coli*, 2) whether those effects were related to cyclic changes in progesterone and estradiol-17β concentrations, and 3) the pattern of change in vena caval concentrations of $PGF_{2\alpha}$ and PGE_2 after intrauterine inoculations. Gilts (n = 5 per group) were assigned to treatments in a 2 × 2 factorial arrangement. Stage of estrous cycle (follicular, d 0 vs. luteal, d 8) and intrauterine inoculation (PBS vs. bacteria) were the main effects. On d 0 or 8 relative to the day of onset of altrenogest-induced estrus, either sterile PBS (10 mL) or a 10-mL suspension of bacteria was injected laparoscopically into the uterus of each gilt.

Experiment 2. The objective for Exp. 2 was to determine whether progesterone induced the changes in the uterine immune system in Exp. 1 that resulted in increased susceptibility to infections after intrauterine bacterial inoculation. Gilts (n = 5 per group) were assigned to treatments in a 2 × 2 factorial arrangement. Ovariectomy (**OVEX**; Sham vs. OVEX) and progesterone treatment (safflower oil diluent [**Oil**] vs. Progesterone) were main effects. All gilts received intrauterine bacterial inoculations.

On d 0 relative to the day of onset of estrus, gilts were ovariectomized or a sham procedure was performed. Immediately after surgery, gilts began receiving i.m. injections of progesterone (10 mg/5 mL) or 5 mL of safflower oil twice daily. On d 8, vena caval catheters were inserted, and the uterus was inoculated with bacteria.

Statistical Analyses. The GLM procedures of SAS (SAS Inst., Inc., Cary, NC) were used to analyze the data. For Exp. 1, the GLM model included terms for stage of estrous cycle, bacteria, stage of estrous cycle × bacteria, gilt nested within stage of estrous cycle × bacteria, time, stage of estrous cycle × time, bacteria × time, and stage of estrous cycle × bacteria × time. Gilt nested within stage of estrous cycle × bacteria was the main plot error term, and the residual was the subplot error term. For Exp. 2, the GLM model included the following independent variables: OVEX, Progesterone, OVEX × Progesterone, gilt nested within OVEX × Progesterone, time, OVEX × time, Progesterone × time, and OVEX × Progesterone × time. Gilt nested within OVEX × Progesterone was the main plot error term, and the residual was the subplot error term.

In the analyses for Exp. 1 and 2, the variance associated with the main plot error term was used to calculate overall SEM associated with main plot variables. When

appropriate, the PDIFF (i.e., a method for comparing all possible least squares means) option in SAS was used to compare individual means.

The shapes of the response curves for unstimulated lymphocyte proliferation were determined for gilts in each treatment group, and then unstimulated activity was used as a covariant for Con A- and LPS-stimulated lymphocyte proliferation. The incorporation data were reported after the adjustment for unstimulated activity, rather than reporting a stimulation index. The reason for this is because a stimulation index represents a percentage change from unstimulated activity, and the index is based on the assumption that the relationship between unstimulated activity and stimulated incorporation is linear, which was not always true for the data from this experiment. The prediction option in SAS was used to generate a new set of data that contained the adjusted values for Con A and LPS. The adjusted values were analyzed with the original GLM model.

Results

Experiment 1

Uterine Infections. All of the gilts inoculated with *A. pyogenes* and *E. coli* on d 8 of the estrous cycle developed uterine infections (sediment volume of 42% and bacterial regrowth), but none of the gilts inoculated with PBS or bacteria on d 0 or with PBS on d 8 developed infections (sediment volume <5% and little to no bacterial regrowth).

Lymphocyte Blastogenic Activity. For unstimulated and Con-A- and LPS-stimulated lymphocyte proliferation, the bacteria × stage of estrous cycle × time interaction was significant ($P < 0.01$; Figure 1). Unstimulated lymphocyte proliferation increased from 0.25 to 0.8 pmol during the first 24 h after inoculation with either PBS or bacteria on d 0 of the estrous cycle (Figure 1). Unlike the PBS-treated group on d 0, unstimulated lymphocyte proliferation in the bacteria-treated group on d 0 returned to basal 48 h after inoculation (Figure 1). After PBS treatment on d 0 of the estrous cycle, unstimulated lymphocyte proliferation remained increased throughout the remainder of the sampling period. Unstimulated lymphocyte activity was less than 0.1 pmol for gilts treated with PBS or bacteria on d 8 of the estrous cycle (Figure 1). After bacterial inoculation on d 0 of the estrous cycle, Con A-stimulated proliferation increased from 1.8 to 3.1 pmol in the first 24 h and remained increased throughout the sampling period (Figure 1). The Con A had little effect on lymphocyte proliferation in the other three treatment groups. Lipopolysaccharide-stimulated lymphocyte proliferation increased from 0.2 to 1.4 pmol during the first 24 h after bacterial inoculation on d 0, and the values had returned to basal by 48 h after inoculation (Figure 1). After intrauterine PBS on d 0 of the estrous cycle, LPS-stimulated lymphocyte proliferation increased and remained so throughout the sampling period (Figure 1).

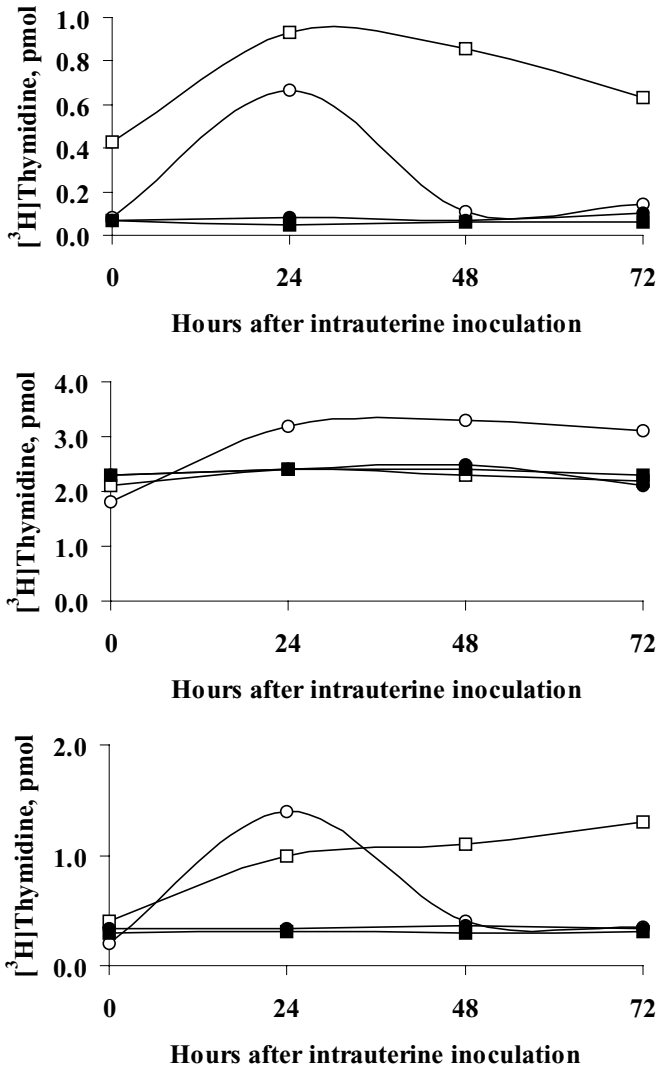


Figure 1. Incorporation of [^{3}H]thymidine into newly formed lymphocytes collected from vena caval blood from gilts (n = 5 per group) that were inoculated with either PBS or bacteria on either d 0 or 8 of the estrous cycle in Exp. 1. The upper panel shows unstimulated lymphocyte proliferation. The middle panel shows concanavalin A (Con A)-stimulated lymphocyte proliferation. The lower panel shows lipopolysaccharides (LPS)-stimulated lymphocyte proliferation. Squares represent PBS treatment. Circles represent bacteria treatment. Open symbols represent treatments administered on d 0 of the estrous cycle. Closed symbols represent treatments administered on d 8 of the estrous cycle. The bacteria × stage of estrous cycle × time interaction was significant ($P < 0.01$) for unstimulated (SEM = 0.04) and mitogen-stimulated lymphocyte proliferation (Con A SEM = 0.1; LPS SEM = 0.05).

Lipopolysaccharide-stimulated lymphocyte activity remained basal after either intrauterine PBS or bacteria on d 8 of the estrous cycle (Figure 1).

Differential White Blood Cell Counts. For neutrophils and lymphocytes, the stage of the estrous cycle × time

and the bacteria × time interactions was significant ($P < 0.01$, Figure 2). The numbers of lymphocytes per 100 WBC in gilts inoculated with PBS or bacteria on d 0 increased ($P < 0.01$) and the numbers of neutrophils decreased ($P < 0.01$). The increase in number of lymphocytes and decrease in number of neutrophils per 100 WBC was maintained over time in the absence of bacterial inoculation on d 0 (Figure 2). However, 72 h after bacterial inoculation on d 0, neutrophil numbers had increased, whereas lymphocyte numbers had decreased somewhat (Figure 2). After inoculation with either PBS or bacteria on d 8, neither neutrophil nor lymphocyte populations changed appreciably over the 72-h sampling period.

For eosinophils, time was significant ($P < 0.05$), and for monocytes, the bacteria × stage of the estrous cycle × time interaction was significant ($P < 0.05$). However, the numerical changes in percentages of each cell type were not striking, and the numbers were close to their means (Figure 2). In all cases, before and after treatment, the numbers of basophils per 100 WBC were too few for meaningful statistical analyses.

Steroids and Prostaglandins. The stage of the estrous cycle × day interaction was significant ($P < 0.01$) for vena caval progesterone (Figure 3). Progesterone concentrations were less in gilts inoculated on d 0 than in gilts inoculated on d 8 (6.3 vs. 52 ng/mL, SEM = 3.4). Progesterone concentrations increased daily in gilts inoculated on either d 0 or 8.

The stage of estrous cycle × day interaction was significant ($P < 0.01$) for vena caval estradiol-17β (Figure 3). Not only were estradiol-17β concentrations greater in gilts inoculated on d 0 than in those inoculated on d 8 (39.8 vs. 11.4 ng/mL, SEM = 3.6), but estradiol increased for the first 48 h after treatment on d 0 and then decreased in the last 24 h of sampling. Changes in estradiol-17β and progesterone were typical of the changes associated with the estrous cycle in pigs and confirmed that gilts were inoculated, as planned, during the follicular or luteal phase of the estrous cycle.

The bacteria × stage of estrous cycle × time interaction was significant ($P < 0.01$) for $PGF_{2\alpha}$. Vena caval $PGF_{2\alpha}$ was greater ($P < 0.01$) in gilts inoculated with bacteria than in gilts inoculated with PBS (0.35 vs. 0.16 ng/mL, SEM = 0.05), and $PGF_{2\alpha}$ concentrations changed with time and in conjunction with stage of the estrous cycle after inoculation (Figure 4).

The bacteria × time interaction was significant for PGE_2 ($P < 0.05$; Figure 4). Overall, PGE_2 concentrations increased and deceased during the sampling period, and concentrations were greater ($P < 0.01$) in bacteria-treated than in PBS-treated gilts (Figure 4). In response to inoculation with bacteria, $PGF_{2\alpha}$ and PGE_2 concentrations increased regardless of the stage of the cycle at the time of inoculation (Figure 4).

Experiment 2

Uterine Infections. All gilts that were exposed to ovarian and/or exogenous progesterone developed uterine

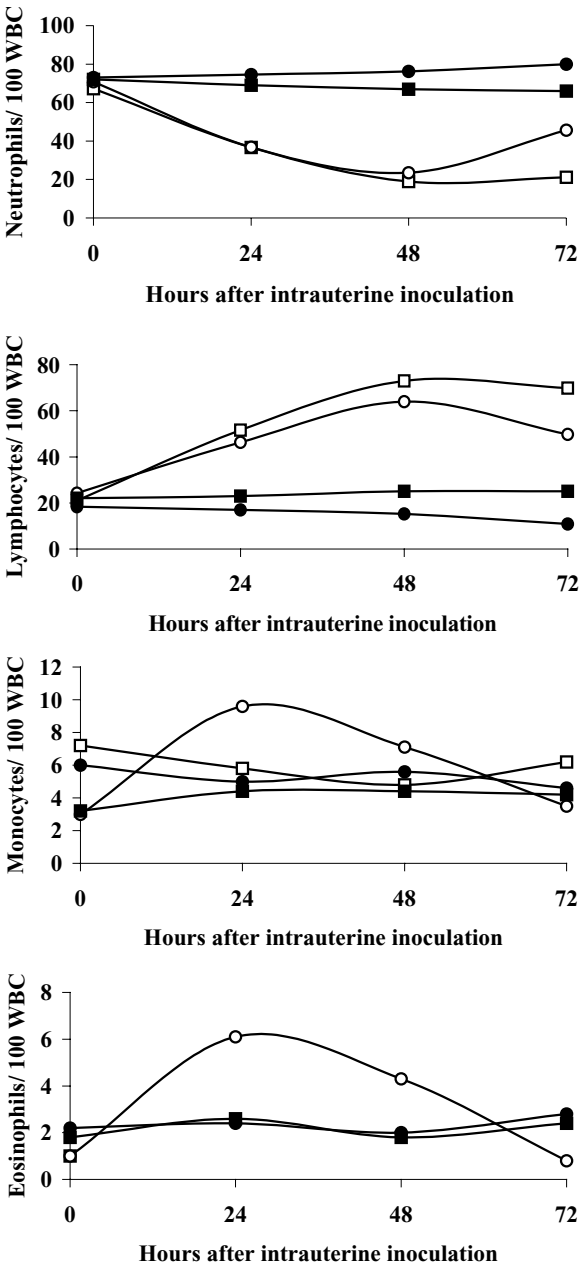


Figure 2. Numbers of neutrophils, lymphocytes, monocytes, and eosinophils per 100 white blood cells (WBC) in Exp. 1. White blood cells were counted in smears of vena caval blood that were collected from gilts (n = 5 per group) given intrauterine inoculations of either PBS or bacteria on d 0 or 8. Squares represent PBS treatment. Circles represent bacteria treatment. Open symbols represent treatments administered on d 0 of the estrous cycle. Closed symbols represent treatments administered on d 8 of the estrous cycle. The y-axis range differs for each WBC type so that the relative differences within WBC type are easier to discern. For neutrophils and lymphocytes, the stage of the estrous cycle × time and the bacteria × time interactions was significant ($P < 0.01$). For eosinophils, time was significant ($P < 0.05$), and for monocytes, the bacteria × stage of the estrous cycle × time interaction was significant ($P < 0.05$). Neutrophil SEM = 3.5; lymphocyte SEM = 3.3; monocyte SEM = 1.1; and eosinophil SEM = 0.95.

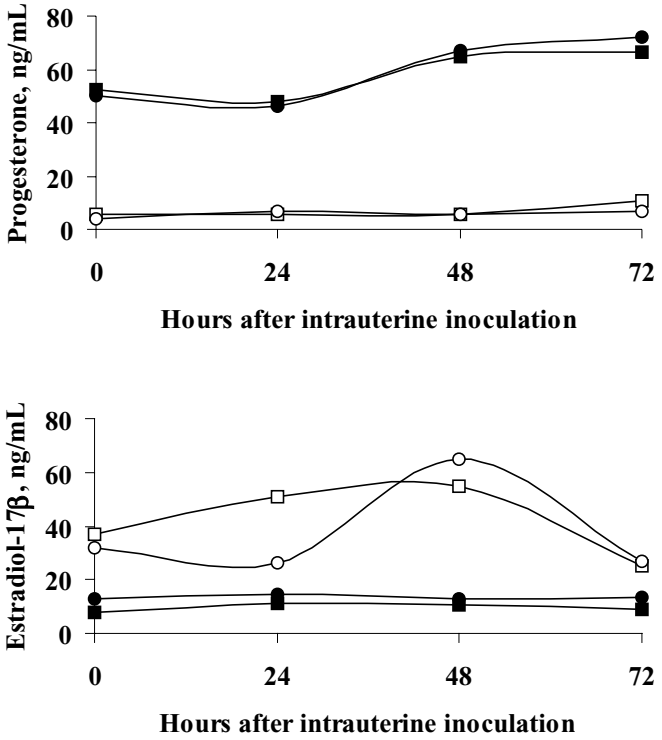


Figure 3. Progesterone and estradiol-17β concentrations in vena caval plasma from gilts (n = 5 per group) that were inoculated with either PBS or bacteria on either d 0 or 8 of the estrous cycle in Exp. 1. Squares represent PBS treatment. Circles represent bacteria treatment. Open symbols represent treatments administered on d 0 of the estrous cycle. Closed symbols represent treatments administered on d 8 of the estrous cycle. For both hormones, the stage of the estrous cycle × day interaction was significant (P < 0.01; progesterone SEM = 3.4; estradiol-17β SEM = 3.6).

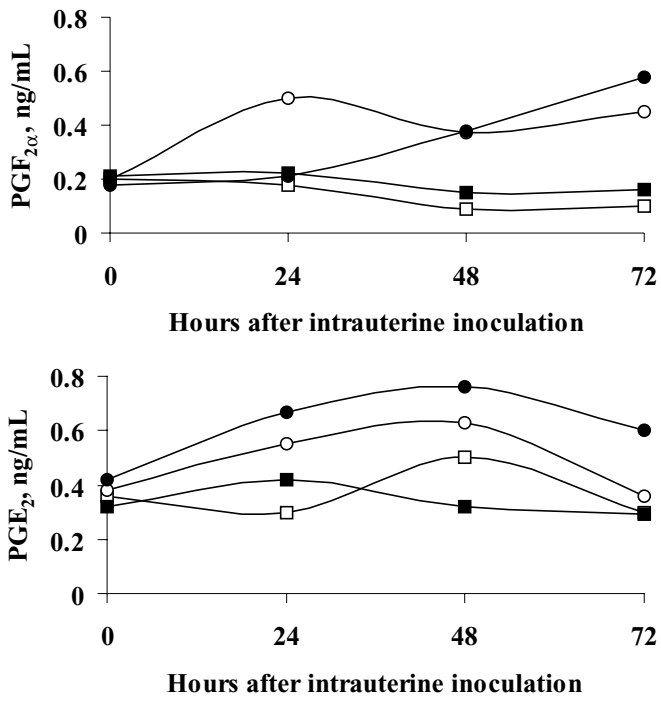


Figure 4. Prostaglandin $F_{2\alpha}$ and PGE_2 concentrations in vena caval plasma from gilts (n = 5 per group) that were inoculated with either PBS or bacteria on either d 0 or 8 of the estrous cycle in Exp. 1. Squares represent PBS treatment. Circles represent bacteria treatment. Open symbols represent treatments administered on d 0 of the estrous cycle. Closed symbols represent treatments administered on d 8 of the estrous cycle. For $PGF_{2\alpha}$, the bacteria × stage of estrous cycle × time interaction was significant (P < 0.01; SEM = 0.03). For PGE_2, the bacteria × time interaction was significant (P < 0.05; SEM = 0.05).

infections (sediment volume of 46% and bacterial regrowth) after intrauterine inoculations with *A. pyogenes* and *E. coli*. Gilts that were not exposed to ovarian and/or exogenous progesterone did not develop uterine infections (sediment volume <5% and slight to no bacterial regrowth). In addition to demonstrating that the experimental model was appropriate for this experiment, this indicates that nonovarian sources (i.e., adrenal) of progesterone had no detectable effect on the uterine response to infectious bacteria.

Lymphocyte Blastogenic Activity. The Progesterone × time and the OVEX × time interactions were significant (P < 0.01) for unstimulated incorporation of [^{3}H]thymidine (Figure 5). Unstimulated incorporation of [^{3}H]thymidine into newly formed lymphocytes increased (P < 0.01) in response to Progesterone (0.09 vs. 0.05 pmol; Progesterone vs. Oil) and decreased in response to OVEX (i.e., removal of a major endogenous progesterone source; 0.06 vs. 0.09 pmol; OVEX vs. Sham).

The main effects of OVEX and Progesterone (i.e., presence or absence of a major source of progesterone) were significant for Con A-stimulated incorporation of [^{3}H]thymidine into newly formed lymphocytes (P < 0.01; Figure 5). In the presence of ovarian and/or exogenous progesterone, Con A-stimulated lymphocyte proliferation was greater than it was in the absence of ovarian and/or exogenous progesterone.

For LPS-stimulated [^{3}H]thymidine incorporation, the OVEX × time and the Progesterone × time interactions were significant (P < 0.01; Figure 5). In the presence of major endogenous and/or exogenous sources of progesterone, LPS-stimulated lymphocyte proliferation was greater (P < 0.05) than it was in the absence of major sources of progesterone.

Differential White Blood Cell Counts. For neutrophils, lymphocytes, monocytes, and eosinophils, the Progesterone × OVEX × time interaction was significant (P < 0.05; Figure 6). The numbers of neutrophils were greater (P < 0.01) in ovary-intact vs. OVEX gilts (72 vs. 58/100 WBC; Figure 6). Progesterone treatment increased (P < 0.05) the number of neutrophils (63 vs. 68/100 WBC; Oil vs. Progesterone; Figure 6). Ovariectomized gilts had greater (P < 0.01) numbers of lymphocytes than did ovary-intact gilts (20 vs 31/100 WBC;

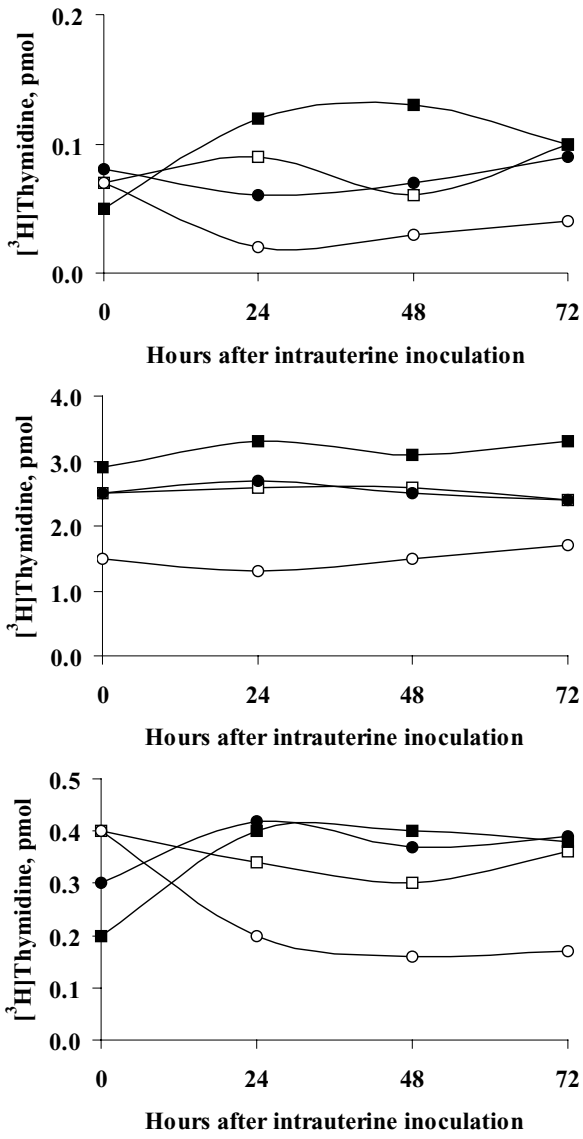


Figure 5. Incorporation of [^{3}H]thymidine into newly formed lymphocytes collected from vena caval blood from gilts (n = 5 per group) that were either sham ovariectomized or ovariectomized on d 0 of the estrous cycle and received either safflower oil diluent or exogenous progesterone in Exp. 2. All gilts were given intrauterine inoculations of bacteria 8 d after the onset of estrus. The upper panel shows unstimulated lymphocyte proliferation. The middle panel shows concanavalin A (Con A)-stimulated lymphocyte proliferation, and the lower panel shows lipopolysaccharides (LPS)-stimulated lymphocyte proliferation. Squares represent sham ovariectomies. Circles represent ovariectomies. Open symbols represent gilts receiving safflower oil diluent. Closed symbols represent gilts receiving progesterone. The progesterone × time and ovariectomy × time interactions were significant ($P < 0.01$) for unstimulated proliferation (SEM = 0.01). The main effects of progesterone and ovariectomy were significant ($P < 0.01$; SEM = 0.1) for Con A-stimulated lymphocyte proliferation. The progesterone × time and ovariectomy × time interactions were significant ($P < 0.01$; SEM = 0.01) for LPS-stimulated proliferation.

Figure 6). The numbers of lymphocytes in ovariectomized oil-treated gilts were increased somewhat at 24 and 48 h after inoculation, but the increases were not remarkable. Numbers of monocytes and eosinophils were increased somewhat in the Sham, progesterone-treated gilts 24 and 48 h after inoculation, but numbers in the other three groups were comparatively constant throughout the sampling period.

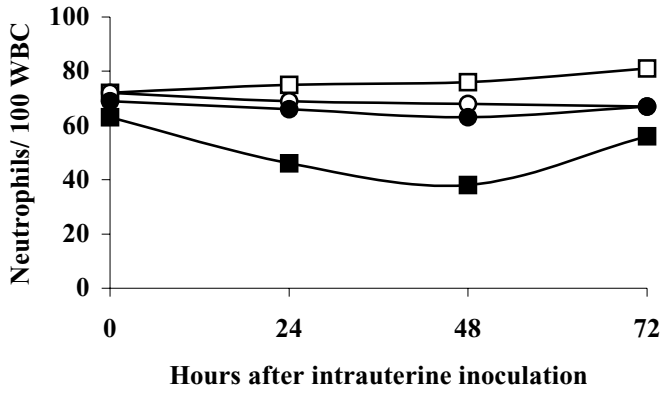
Steroids and Prostaglandins. Ovariectomy and Progesterone affected vena caval progesterone concentrations; they were decreased ($P < 0.01$) in OVEX gilts (13.5 vs. 70 ng/mL; OVEX vs. Sham) and increased ($P < 0.01$) in progesterone-treated gilts (61 vs. 22 ng/mL; Progesterone vs. Oil; SEM = 6.5; Figure 7). Concentrations of estradiol-17β were decreased ($P < 0.01$) in OVEX gilts (10.2 vs. 1.6 ng/mL; OVEX vs. Sham; SEM = 2.3), but not in Progesterone-treated gilts, indicating that progesterone treatment did not affect estradiol-17β (Figure 7).

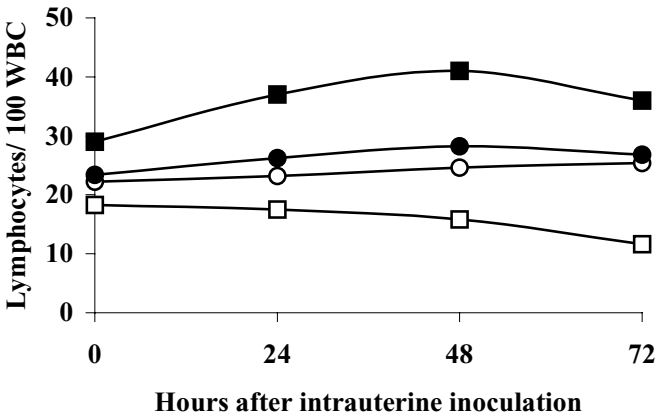
Vena caval $PGF_{2\alpha}$ concentrations changed ($P < 0.01$) with time after inoculation, but the changes were not striking (Figure 8). Treatments did not affect vena caval PGE_2 concentrations, time was not significant, and none of the interactions were significant. The average PGE_2 concentration was 0.38 ng/mL (SEM = 0.34).

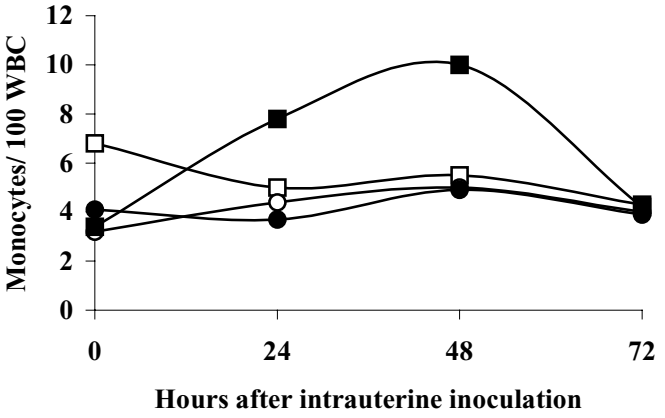
Discussion

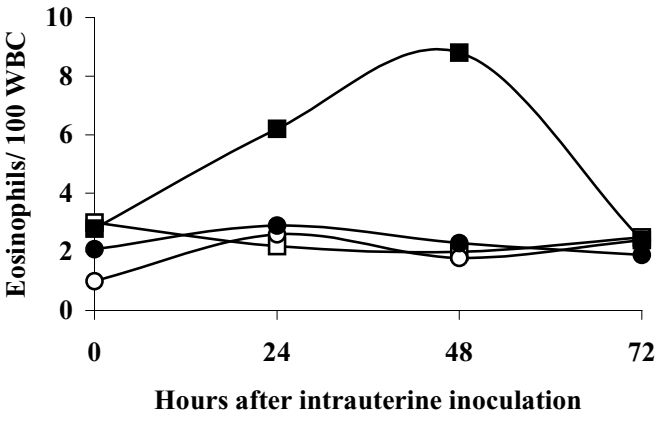
The uterine response to *E. coli* and *A. pyogenes* during estrus and the luteal phase of the estrous cycle in gilts was similar to the uterine response reported for sheep and cattle (Ramadan et al., 1997; Dhaliwal et al., 2001; Seals et al., 2002). After intrauterine bacterial inoculation during the luteal phase (d 8), gilts were susceptible to uterine infections, and during estrus (d 0), gilts were resistant to uterine infections. Specifically, immune functions measured as lymphocyte proliferation and white blood cell changes and confirmed with the establishment or lack thereof of uterine infections were up-regulated at estrus (i.e., when progesterone was decreased and estrogen was increased) and down-regulated during the luteal phase and/or when progesterone was increased (Exp. 1 and 2).

At estrus, unstimulated and Con A- and LPS-stimulated lymphocyte proliferation increased in response to intrauterine injection of PBS or bacteria. Increases in unstimulated and LPS-stimulated lymphocyte proliferation were maintained for several days after inoculation with PBS, but proliferation after bacterial inoculation returned to basal after a 24-h increase. By contrast, Con A-stimulated lymphocyte proliferation increased after intrauterine inoculation with bacteria on d 0 and remained increased for 72 h after inoculation. Intrauterine inoculation (i.e., PBS or bacteria) on d 8 did not change unstimulated or mitogen-stimulated lymphocyte proliferation. These results indicate that, in the presence or absence of an intrauterine bacterial challenge, lymphocyte activity fluctuates during the estrous cycle in gilts. Lymphocyte activity seems to increase









at estrus and decrease during the luteal phase. After bacterial challenge at estrus, this pattern held true; however, there was a shift in proliferative activity. Rather than an increase in total lymphocyte proliferation, T lymphocytes seemed better able to proliferate. Changes in circulating pools of white blood cells corresponded to changes in proliferative activity of lymphocytes at estrus and throughout the follicular phase and in response to bacterial inoculation. On d 0, populations of neutrophils were greater than populations of lymphocytes. However, during the first 72 h (i.e., corresponding to the follicular phase of the estrous cycle) after intrauterine inoculation with either PBS or bacteria, populations of circulating lymphocytes increased, whereas neutrophils decreased. The shift in white blood cell populations may represent a shift in immune cell "preparedness." That is, during the follicular phase, the pool of available circulating lymphocytes seems to increase, and the pool of available circulating neutrophils seems to decrease. The increase in numbers of vena caval lymphocytes may reflect the movement of immunocompetent cells from secondary lymphoid tissues into blood (Tizard, 1996). The decrease in circulating neutrophils may be attributed to movement of neutrophils out of circulation and into the uterus (Seals et al., 2002), where they might manage pathogenic organisms that enter the uterus during mating. The reduction of neutrophils in circulation during the follicular phase may be a sign of uterine preparedness for bacterial invasion (Killingbeck and Lamming, 1963; Carson et al., 1988).

In addition to the influence of steroids on the uterine immune system, prostaglandins are known to be potent immunoregulatory molecules (Razin et al., 1978, Steffan et al., 1984; Paisley et al., 1986). In response to uterine bacterial inoculation on d 0 or d 8, vena caval $PGF_{2\alpha}$ and PGE_2 increased (Exp. 1). Increases in vena caval $PGF_{2\alpha}$ are consistent with increases reported for cows and sheep with uterine infections (Del Vecchio et

Figure 6. Numbers of neutrophils, lymphocytes, monocytes, and eosinophils per 100 white blood cells (WBC) in Exp. 2. White blood cells were counted in smears of vena caval blood that was collected from gilts (n = 5 per group) receiving either sham ovariectomies or ovariectomies and either safflower oil or exogenous progesterone. All gilts were given intrauterine inoculations of bacteria 8 d after the onset of estrus. Squares represent sham ovariectomies. Circles represent ovariectomies. Open symbols represent gilts receiving safflower oil diluent. Closed symbols represent gilts receiving progesterone. The y-axis range differs for each WBC type so that the relative differences within WBC type are easier to discern. For neutrophils, lymphocytes, monocytes, and eosinophils, the progesterone × ovariectomy × time after inoculation interaction was significant ($P < 0.05$). Neutrophil SEM = 1.9; lymphocyte SEM = 1.4; monocyte SEM = 0.8; and eosinophil SEM = 0.8.

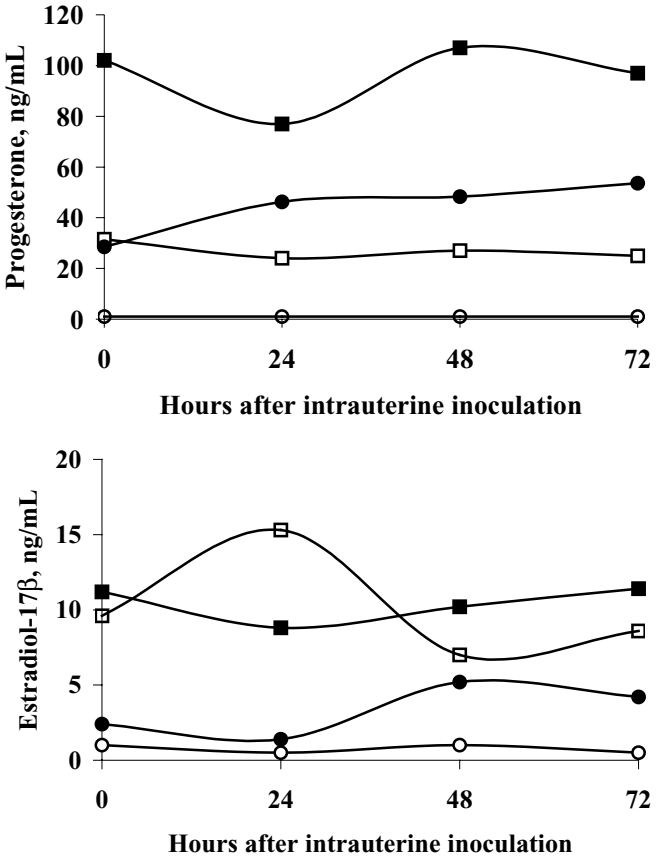


Figure 7. Concentrations of progesterone and estradiol-17β in vena caval blood collected from gilts (n = 5 per group) that were either sham ovariectomized or ovariectomized on d 0 of the estrous cycle and received either safflower oil diluent or exogenous progesterone in Exp. 2. All gilts received intrauterine inoculations of bacteria on d 8 of the estrous cycle. Squares represent sham ovariectomies. Circles represent ovariectomies. Open symbols represent gilts receiving safflower oil diluent. Closed symbols represent gilts receiving progesterone. Ovariectomy decreased ($P < 0.01$) progesterone concentrations (13.5 vs. 70 ng/mL; ovariectomy vs. sham; overall SEM = 6.5), and exogenous progesterone increased ($P < 0.01$) progesterone concentrations (61 vs. 22 ng/mL; progesterone vs. safflower oil). Ovariectomy decreased ($P < 0.01$) estradiol-17β concentrations (10.2 vs. 1.6 ng/mL; ovariectomy vs. sham; SEM = 2.3).

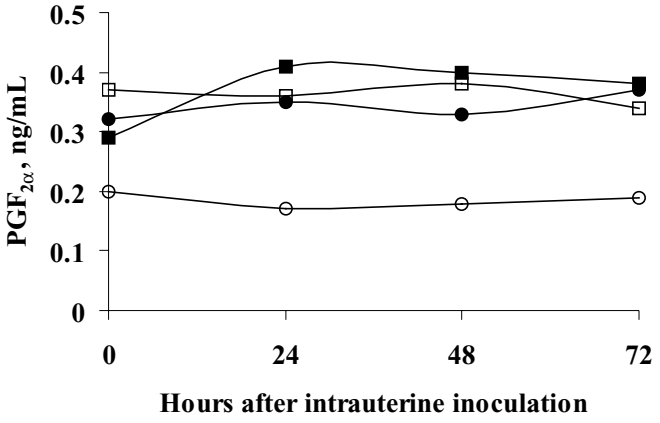


Figure 8. Concentrations of $PGF_{2\alpha}$ in vena caval blood collected from gilts (n = 5 per group) that were either sham ovariectomized or ovariectomized on d 0 of the estrous cycle and received either safflower oil diluent or exogenous progesterone in Exp. 2. All gilts received intrauterine inoculations of bacteria on d 8 of the estrous cycle. Squares represent sham ovariectomies. Circles represent ovariectomies. Open symbols represent gilts receiving safflower oil diluent. Closed symbols represent gilts receiving progesterone. Concentrations of $PGF_{2\alpha}$ changed with time ($P < 0.01$; SEM = 0.06).

al., 1992; Ramadan et al., 1997). We assume that the increase in $PGF_{2\alpha}$ is a result of the inflammation that is normally associated with infections (Tizard, 1996). Indeed, the continued increase in $PGF_{2\alpha}$ concentrations as the infection developed during the luteal phase is consistent with our assumption. Increases in vena caval PGE_2 were more pronounced during the luteal phase, and this may contribute to the decreased chemotaxis of neutrophils (i.e., lack of reduction in numbers of circulating neutrophils/100 WBC) and proliferation of lymphocytes (Garzetti et al., 1998).

In cattle and sheep, susceptibility to uterine infections during the luteal phase is thought to be predominantly mediated through the effects of progesterone on the uterine environment (for review, see Dhaliwal et al., 2001). After establishing that gilts, like cattle and sheep, exhibit a cyclic susceptibility and resistance to uterine infections that correlates with the estrous cycle, we conducted Exp. 2 to determine whether the cycle of susceptibility and resistance was linked directly to the presence or absence of major sources of progesterone. In Exp. 2, if gilts were not exposed to progesterone, they were resistant to infections, whereas gilts that were exposed to exogenous or endogenous progesterone were susceptible to infections. Because ovariectomy reduced the influence of other steroids and ovarian factors, the exogenous progesterone treatment permitted us to relate the increased susceptibility to infection directly to the influence of progesterone.

Endogenous and exogenous progesterone after intrauterine bacterial inoculation increased unstimulated, Con A-stimulated, and LPS-stimulated lymphocyte proliferation compared with that for gilts that were ovariectomized and received safflower oil diluent. However, based on previous work with sheep, these results are the opposite of what we expected (Ramadan et al., 1997). These unexpected differences may be due to the lack of ovarian influences on lymphocyte populations after ovariectomy. This may indicate that steroidal control over the uterine response to infectious bacteria is not due entirely to the presence or absence of ovarian progesterone, but rather to the ratio of progesterone

to estrogen or to a period of estrogen priming before progesterone dominance. In any case, the prolonged follicular phase and prolonged period of estradiol production in gilts, compared with cattle and sheep, may play a greater role in modulating the ability of the uterus to clear bacterial infections than seems to be the case in cattle and sheep (Wuttke et al., 1997; Kaeoket et al., 2001).

The changes in circulating white blood cell populations revealed a possible explanation for the unexpected lymphocyte proliferation results. In the absence of ovarian and/or exogenous progesterone, neutrophil populations decreased in response to bacterial infusion without a significant increase in lymphocyte populations. This seems to indicate that, in the absence of a major steroid influence, a rapid short-term neutrophil response clears the infection. This also supports the idea that progesterone in gilts does not necessarily impede lymphocyte functions as much as it impedes neutrophil functions.

In the presence of ovarian and/or exogenous progesterone in Exp. 2, $PGF_{2\alpha}$ increased after bacterial exposure. As in Exp. 1, increases in $PGF_{2\alpha}$ were most pronounced in the groups with the most severe infections, indicating that the increase in $PGF_{2\alpha}$ is associated with the ensuing metritis. These results parallel similar results for sheep and cows (Del Vecchio et al., 1992; Ramadan et al., 1997; Seals et al., 2002).

Previous reports have documented the cyclic resistance and susceptibility to uterine infections in cows, sheep, and rabbits (Black et al., 1953a,b; Rowson et al., 1953; Ramadan et al., 1997). That is, the resistance to uterine infections in these animals is greater at estrus than during the luteal phase of the estrous cycle (Black et al., 1953a,b; Rowson et al., 1953; Ramadan et al., 1997). This phenomenon has been related to changes in steroid hormone profiles and the subsequent changes in white blood cell behavior throughout the estrous cycle. Unlike results for ruminants, previous research with pigs indicates that some strains of bacteria are able to survive the follicular phase and multiply, leading to infections during the luteal phase that are associated with increased embryonal death (Scofield et al., 1974). Neither Exp. 1 nor 2 was designed to test the hypothesis implied from Scofield et al. (1974). However, our inability to detect significant regrowth of bacteria at the end of the follicular phase indicates that the strains of bacteria used in this study did not survive the follicular phase. Based on the results from this study, changes in exogenous or endogenous progesterone regulate the ability of the uterus to clear bacterial infections through regulation of lymphocyte and neutrophil functions.

Implications

Uterine infections are a major problem in ruminants, but the extent of the problem in swine is not clear. However, one may surmise that, because of management conditions and syndromes with uterine infections as a component, uterine infections are a major problem in the swine industry. Gilts seem to be an ideal model for studying uterine infections. Indeed, this research indicates that changes in steroid concentrations, particularly increases in progesterone, regulate white blood cell functions and decrease the ability of the uterus in gilts to resist infections. Infection-induced increases in uterine prostaglandin production may promote the movement of white blood cells into the uterus, where they might control the pathogens. Thus, we believe that appropriate prostaglandin treatments may be useful for modulating the ability of the uterus to clear infections and decreasing the incidence of uterine infections in pigs.

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